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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|---|---|---|---|
| 09/365,677 | 08/02/99 | LAM | P 16285-11.00U |

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TWO EMBARCADERO CENTER
EIGHTH FLOOR
SAN FRANCISCO CA 94111-3834

| EXAMINER | |
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| DRABIK,C | |
| **ART UNIT** | **PAPER NUMBER** |
| 1633 | 4 |

**DATE MAILED:** 06/20/01

**Please find below and/or attached an Office communication concerning this application or proceeding.**

**Commissioner of Patents and Trad marks**

| Office Action Summary | Application No. 09/365,677 | Applicant(s) LAM ET AL. | |
|---|---|---|---|
| | Examiner Christopher Drabik | Art Unit 1633 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) 1-17 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-17 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

**Attachment(s)**

15) ☐ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

## DETAILED ACTION

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1-14 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Independent claims 1,4,7, 8, 11 and 14 recite the phrase: "derivative of benzyl esterified hyaluronic acid. The claims are indefinite because the term "derivative" only denotes a source or starting material and does not clearly denote the end product to be utilized in the claim. Claims 2,3,5,9,10, 12 and 13 depend from the independent claims 1,4,7, 8, 11 and 14 and hence are also rejected based on the limitation of the independent claims.

Claims 1-6 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 1 and 4 recite a substratum with a layer of fibroblasts which is "at least sub-confluent." A substratum with no cells is at least sub-confluent. Claims 1 and 4 are vague and indefinite because it is unclear whether fibroblasts are, or are not, growing on the biosynthetic substratum. Claims 2 and 3 depend from claim 1. Claims 5 and 6 depend from claim 4., Claims 2, 3, 5 and 6 are rejected based upon the limitation of the claims from which they depend.

Claims 2, 3, 5, 6, 9, 10, 12 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 2, 5, 9 and 12 recite an allogenic dermal fibroblast. Claims 3, 6, 10, and 13 recite an autologous dermal fibroblast. The claims are vague and indefinite because it is unclear to what or whom the cells are autologous or allogenic.

Claims 4-6 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 4 recites "... a first basal side..." and subsequently "... a second upper side.." It is unclear from the claim how many basal sides and /or upper sides constitute the said biosynthetic substratum. Claims 5 and 6 depend from claim 4.

Claims 11-13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter whichapplicant regards as the invention. Claim11 recites "... a first basal side..." and subsequently "... a second upper side.." It is unclear from the claim how many basal sides and /or upper sides constitute the said biosynthetic substratum. Claims 12 and 13 depend from claim 11.

Claim15 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 15 recites "...allowing a sufficient time to form a vascularized wound bed...' It is unclear from the claim what a period is a "sufficient time" for vascularization. The claim is therfeore vague and indefinite.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1 and 8 rejected under 35 U.S.C. 102(b) as being clearly anticipated by Della Valle et al US Patent 5,658,331.

Claim 1 is drawn to a method of cultivating graftable skin involving the co-culture of fibroblasts and keratinocytes on a biosynthetic substrate and wherein said substrate is a benzyl derivative of hyaluronic acid. Claim 8 is drawn to a graftable skin material produced by the method of claim 1.

The nature of the invention claimed in Claims 1 and 8 is a means for in vitro production of skin which may be used for transplantation techniques. It should be noted that the phrase "target donor patient" does not explicitly mean that the keratinocytes to be used are from the same patient to be grafted, but rather the scope of the claim also

includes other target donors. Co-culturing of keratinocytes with fibroblasts to achieve better keratinocyte growth is well established in the art, the examples of such a technique date to at least the mid 70's. The use of hyaluronic acid (HA) and specific derivatives of hyaluronic acid for the culturing of keratinocytes is also well established in the art. Biosynthetic substrates comprising benzyl esterified derivatives of hyaluronic acid were commercially available at the time of applicants filing date and have been specifically marketed for the use of culturing keratinocytes for the production of graftable skin. Indeed, applicants disclose the use of Laserskin, a hyaluronic acid membrane available from Fidia Advanced Biopolymers, in the specification.

Della Valle et al disclose a bio-compatible artificial skin and a method for the production of said artificial skin employing a benzyl-esterified HA membrane. The method involves the deposition of fibroblasts on the HA membrane followed by the addition of keratinocytes and subsequent co-culturing of the keratinocytes and fibroblasts. (See columns 4 and 5, Example 3). The artificial skin is disclosed for use in treating lesions of the body surface (See claim 1). Hence, the limitations of Claims 1 and 8 of the instant application are clearly anticipated by the disclosure of Della Valle et al.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 2, 3, 9 and 10 rejected under 35 U.S.C. 103(a) as being unpatentable over Della Valle et al in view of Hansbrough. (JAMA(1989) 262: 21225-2130)

Claims 2 and 3 are drawn to methods of cultivating a graftable skin material wherein said skin comprises autologous or allogenic fibroblasts. Claims 9 and 10 are drawn to graftable skin material wherein the graftable skin comprises autologous or allogenic fibroblasts. Della Valle et al teach the use of a benzyl esterified biosynthetic substratum incorporating keratinocytes seeded over a layer of mouse 3T3 fibroblasts for the production of graftable skin. Della Valle does not teach the use of allogenic or autologous fibroblasts in the method.

The utility of using autologous or allogenic cells for the purpose of grafting, would be prima facie obvious to one skilled in the art based on the fact that these types of cells are best suited for the avoidance of immune response and hence graft rejection. The use of allogenic cells, rather than the use of autologous cells might be utilized for several obvious reasons, for example, the harvesting of autologous cells from a patient may be impractical or not possible.

Further, methods of cultivating a graftable skin material comprising the growth of keratinocytes in the presence of autologous fibroblasts is well documented in the art. For example, Hansbrough et al disclose a cultivated skin material comprising a autologous fibroblasts co-cultured with keratinocytes in a collagen glycoaminoglycan matrix (see page 2125-21226, Materials and Methods)

Claim 4 – 6 and 11-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Della Valle et al in view of Cooper et al." (J Invest Dermatol, (1993) 110:811-819, Hansbrough et al and Meyers et al (J Burn Care Rehabil, (1997) 18:214-22)

Claims 4 – 6 are drawn to methods of cultivating a graftable skin said graftable skin comprising a biosynthetic substrates of benzyl-esterified hyaluronic acid upon which keratinocytes and fibroblasts are grown. Claims 11-13 are drawn to graftable skin materials having apparently two sides comprising on a first side keratinocytes grown upon a layer of fibroblasts and on a second side only fibroblasts The nature of the invention is the generation of a skin-like material for the use in grafting techniques. An essential element of claims 4-6 is the co-culture of keratinocytes and fibroblast cells on a biosynthetic substrate. The limitations of claim 5 and 6 recite that the fibroblast are allogenic or autologous.

It is well known in the art that the presence of a dermal layer consisting in part of fibroblasts contributes to improved graft "take rates." For example, Cooper et al write: "In skin substitutes, epidermal growth has been increased with the addition of viable fibroblasts...human keratinocytes alone on collagen glycosaminoglycan had poor 'take' on athymic mice, and that the addition of human fibroblasts more than doubled the acceptance and persistence of the grafts on mice(see page 817, 1st column ). In the experiments of Cooper et al they compare the engraftment of composite skin consisting

of keratinocytes and fibroblasts to that of epidermal sheet grafts consisting of cultured keratinocytes alone. For the composite skin, fibroblasts were seeded in a collagen matrix and later keratinocytes seeded on a non-porous side of the composite. While Cooper et al does not teach the use of hyaluronic acid membrane, Cooper et al teaches that the inclusion of fibroblasts in graftable material increases the production of collagen in vitro, results in the production of laminin at the appropriate dermoepidermal junction in vivo and improves the quality of the dermoepidermal junction compared to keratinocyte sheet grafts. Cooper et al conclude: "...addition of fibroblasts in the graft along with keratinocytes, enhances formation of basement membrane proteins, which improves attachment of the epidermal layer" (see page 817-818). The results presented in this paper are an extension of work published in Hansbrough et al in which collagen aminoglycan membranes were used to autograft cultured fibroblasts and keratinocytes in burn patients.

Myers et al teaches the use of a hyaluronic acid membrane for the delivery of cultured keratinocytes. The method involves the culturing of autologous keratinocytes on a perforated hyaluronic membrane in the presence of irradiated, non-proliferating fibroblast cells . Experiments described in Meyers et al include the comparison of grafting in the presence or absence of a dermal layer. Dermal layers in wound areas were generated by autografting of de-epidermalised dermis (which consists essentially of fibroblasts.) The data of Meyers' indicates that the best results were achieved with keratinocytes cultured on hyaluronic acid membranes and grafted

onto wounds which contained a dermal layer, i.e. which had fibroblasts present. (see Materials and Methods section, pages 215-218 and page 218, Table 2).

It would be readily apparent to one of skill in the art that a reasonable application of hylauronic acid membranes for grafting would incorporate the teachings of Cooper et al - indicating that the co-culturing of live fibroblasts with keratinocytes in cultivated skin material provides advantages over keratinocytes alone - and the teachings of Meyers et al – which points to the need of fibroblast (dermal) cells for better graft formation. Both point to a crucial interaction of fibroblasts and keratinocytes in graft formation. Hansbrough et al teach the use of autologous fibroblasts and keratinocytes in a collagen-glycosaminoglycan matrix anticipating the use of a biosynthetic substratum containing autologous and allogenic cells. (see page 2125, column 3 – Materials and Methods). Given the directionality of the dermo-epidermal junction it would be prima facie obvious to culture keratinocytes and fibroblasts as disclosed in claim 4 with one side of the membrane facing the developing dermis and consisting of fibroblasts and a second side of keratinocytes underlaid with fibroblasts. The culturing of keratinocytes over viable fibroblasts on one face of the membrane is an obvious extension of the use of 3T3 feeder cells and further anticipates the need for a good dermo-epidermal junction.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Della Valle et al further in view of Rennenkampf et al (Surgery (1996) 120:16-22).

Della Valle teaches the use of hyaluronic membranes for the growth of keratinocytes. The method of Della Valle et al utilized a feeder layer of irradiated 3T3 fibroblast cells. Rennekampf et al teach the growth of keratinocytes in the absence of fibroblast feeder cells. (see page 17 2nd col). One of skill in the art could clearly apply the technique of Rennekampf et al to the culturing of keratinocytes on hyaluronic membranes with a reasonable chance of success. Motivation for combining the techniques can be found for example in situations in which 3T3 cells are too immunogenic for engraftment techniques.

Claims 15-17 rejected under 35 U.S.C. 103(a) as being unpatentable over Orgill et al ( US Patent 5,489,304), Zacchi et al (J Biomed Mater Res (May 1998) 40:187-194), Meyers et al (J Burn Care Rehabil (1997) 18:214-22) Della Valle et al and Hansbrough et al.

Claims 15 –17 are drawn to a method of grafting a graftable skin material, said method involving the use of a layer of collagen-glycoaminoglycan , followed by the application of a biosynthetic substratum comprising at least a layer of keratinocytes. The nature of the invention has to do with a skin grafting methodology involving the formation of a dermal layer facilitated by the application of a collagen-glycoaminoglycan substrate upon which a subsequent layer of cultivated skin material (comprising epithelial cells) is applied. The result of this procedure is a skin graft involving formation of both a new dermis based on vascularization and dermal cell colonization of the collagen matrix and a new epidermis derived from autologous keratinocytes. It should

be noted that the broadest scope of the claims encompasses the application of autologous keratinocytes without any supporting material, i.e. cultured keratinocytes without Laserskin, since keratinocyte sheets can grow to thicknesses of more than one cell. The collagen-glycosamnioglycan substrate referred to in claim 15 includes, commercially available products, for example Integra. Claims 16 and 17 recite that the skin material comprise a biosynthetic substratum comprising a layer of fibroblasts and keratinocytes. Claim 17 recites that fibroblast layers are present on both sides of said biosynthetic substratum.

Orgill et al teach the use of a collagen glycosaminoglycan matrix (CG-matrix) for generating a dermal layer in patients suffering from burn injuries. The method of Orgil et al involves the generation of a "neodermis" facillitated by the placement of a CG-matrix followed by the engraftment of a subsequent layer of cultured epithelial cells (CEA – comprising keratinocytes). (see column 4, line 9 – column 6, line 26 Orgil et al explain that the use of CEA (which comprise keratinocytes) in the absence of a dermal substrate suffers from poor take rates (see column 2, lines 45- line 68 Orgil et al does not teach the use of cultured keratinocytes supported by a biosynthetic substratum.

Zacchi et al teaches the use of hyaluronic acid substrates for the cultivation of fibroblasts and keratinocytes. Zacchi et al in commenting on the state of the art disclose:

> **"Even though keratinocyte culture techniques have improved with the use of potent stimulators of their proliferation and colony forming capacities, resulting in the development of confluent keratinocyte laminae up to a thickness of 10-15 cells their clinical use is often unsatisfactory. However, it is now widely accepted that the presence**

**of a scaffold supporting the keratinocyte layers might overcome several of the problems, such as fragility, handling, contraction and, with an appropriate preparation of the wound bed, improve the 'take' rate, otherwise unpredictable before grafting."(see page, column )**

This disclosure indicates that the use of keratinocytes on a support, such as a hyaluronic acid membrane, is beneficial in the grafting of keratinocytes.

Zacchi et al does not teach two step grafting techniques in which a neodermis is initially formed.

Myers et al teaches the use of a hyaluronic acid membrane for the delivery of cultured keratinocytes. The method involves the culturing of autologous keratinocytes on a perforated hyaluronic membrane in the presence of fibroblast cells . Experiments described in Meyers et al include the comparison of grafting in the presence or absence of a dermal layer. In addition, grafting of cultured keratinocytes with and without a membrane support are compared. (see Materials and Methods section, pages 215-218). The results of Meyers indicate that the best results were achieved with keratinocytes cultured on hyaluronic acid membranes and grafted onto wounds which contained a dermal layer (see page 218, Table 2). Meyers et al state:

**" Hyaluronic acid promotes cell migration, and proliferation. It is a major extracellular matrix component of foetal skin, with an ontogenic transition in its metabolism during foetal development suggesting a role for hyaluronic acid for scarless foetal wound healing. Hyaluronic acid degradation products are angiogenic. All of these make hyaluronic acid an appropriate substrate for a membrane delivery system... It is clear,**

**however, that this membrane does not replace live autologous dermis in the promotion of graft take...**

**Clinical take of keratinocyte grafts relies in the first instance on the provision of a dermal wound bed, ideally live and autologous.**

**Membrane delivery systems of this sort offer the opportunity, in combination with a dermal wound bed, to achieve superior keratinocyte take with large expansion rates, easier handling and greater clinical flexibility. (page 221)**

Meyers et al does not teach the generation of a dermal wound bed using a collagen-glycoaminoglycan matrix.

Hansbrough et al teaches a biosynthetic substrate useful for the co-culturing of autologous fibroblasts and keratinocytes. The biosynthetic substrate is used for grafting on to wounded skin. Hansbrough et al does not teach the use of hyaluronic acid for the co-culture of fibroblasts.

Della Valle et al teach the co-culture of irradiated 3T3 fibroblasts with keratinocytes on hyaluronic membranes. Della Valle does not teach the co-culture of autologous or allaogenic cells.

It would be prima facies obvious to one of skill in the art to combine the teaching of Orgill regarding the use of an artificial dermis (comprising e.g Integra) in combination with the teachings of Zacchi et al and Meyers et al concerning the use of a hyaluronic supporting membrane and the need for an adequate dermal wound bed. The teachings of Della Valle et al and Hansbrough et al point to the obviousness of co-

culturing fibroblasts and keratinocytes on hyaluronic acid membrane and further the use of autologous fibroblasts and keratinocytes in co-cultures on a biosynthetic substratum.

Given the reasoning set forth in the previous obviousness type rejection involving the use of autologous or allogenic cells and the specific growth of the cells regarding there spatial relation (keratinocytes over fibroblasts over membrane, fibroblasts) and the importance of the formation of a dermal-epidermal juncture it would be apparent to one of skill in the art to combine the foregoing methodologies for grafting.

***Conclusion***

No claims of the instant application are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher Drabik whose telephone number is 703-605-1156. The examiner can normally be reached on Monday-Friday from 9am to 5pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Clark, can be reached on 703- 305-4051. The fax phone number for the organization where this application or proceeding is assigned is 703-308-4242.

Inquiries of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-1234. Questions regarding review of formality issues may be directed to Kim Davis, the patent analyst assisting in this application. She may be reached at 703-305-3015.

Deborah J. Clark

**DEBORAH J. R. CLARK**
**SUPERVISORY PATENT EXAMINER**
**TECHNOLOGY CENTER 1600**